REMARKS

Reconsideration of the rejections set forth in the Office action mailed April 10, 2006 is respectfully requested. Claims 1-16 are pending in the application. Claims 1-14 are under examination. Claims 15-16 are currently withdrawn from consideration.

I. The Claims

Applicants note that step (d) of claim 1 is misquoted on pages 3, 6, and 8 of the Office Action (as in the previous Office Action) as "labeling the polynucleotide" rather than "labeling the oligonucleotide tag".

II. Rejections under 35 U.S.C. §102(b)

The rejection of claim 1 and dependent claims 4, 6-8 and 10 under 35 U.S.C. §102(b), as being anticipated by Wong (U.S. Patent No. 5,935,793), was reiterated verbatim from the previous Office Action. This rejection is respectfully traversed for the following reasons.

The applicants refer to the response filed August 9, 2006 for a discussion of the claimed invention and a general discussion of the cited reference.

In the previous response (pages 9-10), the applicants stated that the claimed method differs from that taught by Wong, in one aspect, in that the claim steps of "(b) generating a size ladder of polynucleotide fragments" and "(c) separating the polynucleotide fragments into size classes" both precede the step of "(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides..."

Applicants further stated that, in Wong, the step of "generating a size ladder of polynucleotide fragments" (i.e., Sanger sequencing or Maxam-Gilbert sequencing) follows, or is concomitant with, labeling (of the extension primer or terminating nucleotide in Sanger sequencing, or the entire polynucleotide in Maxam-Gilbert sequencing). The step of "separating the polynucleotide fragments into size classes" in Wong (i.e. separating the Sanger or Maxam-Gilbert sequencing fragments by electrophoresis) also follows the step of labeling in Wong.

The applicants also noted, in the previous response (bottom of page 10) that:

One exception is described at column 22, lines 13-22 of Wong, where "primer-tag-primer"

regions of separated fragments are labeled during PCR amplification, "to allow ready detection of the amplified tag sequences". However, the label in this case is the same for every amplified species, contrary to the applicants' claim, which recites "labeling the oligonucleotide tag of each polynucleotide fragment *according to the identity of one or more nucleotides at an end of such polynucleotide fragment*".

In the present Office Action (page 11), the Examiner states that Wong "expressly teaches labeling that occurs after steps b) and c)", referring to the same passage noted by applicants above (column 22, lines 13-25). However, as stated by applicants in the previous response, 'the label in this case is the same for every amplified species [since the label is on a PCR primer], contrary to the applicants' claim, which recites "labeling the oligonucleotide tag of each polynucleotide fragment *according to the identity of one or more nucleotides at an end of such polynucleotide fragment*".' Therefore, this labeling process in Wong does not meet the limitations of the claim.

In view of the above comments, the reference does not disclose all of the elements set out above in claim 1, and arranged as in this claim. Claim 1 and its dependent claims therefore are not anticipated by this reference, and the applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

III. Rejections under 35 U.S.C. §103

The rejection of claim 1 and dependent claims 2-12 and 14 under 35 U.S.C. §103(a), as being unpatentable over Brenner (U.S. Patent No. 5,763,175) in view of Wong, cited above, was reiterated verbatim from the previous Office Action. The rejections are respectfully traversed in light of the following remarks.

The applicants refer to the response filed August 9, 2006 for a discussion of the claimed invention and a general discussion of cited reference Wong.

The following general discussion of Brenner is reproduced from the previous response:

> Brenner is directed to a method of simultaneous sequencing of polynucleotides. As shown in the embodiment of Fig. 2, multiple sets of "S primers" are applied to a construct made up of an S primer binding site (22), the target polynucleotide (20), a tag (16) flanked by cleavage sites (14 and 18), and a second primer binding site (12). The S

primers are provided in sets of four, as shown in the Figure, where within each set one nucleotide is either A, G, C, or T. The different sets of S primers (shown as Sets 1, 2, 3, ...k) differ in how far this varying nucleotide, which is the site of sequence interrogation, extends into the target polynucleotide.

As described e.g. at column 13, lines 14-26 and column 20, lines 26-55 of the patent, separate PCR reactions are carried out with the different primer sets. For a given target polynucleotide, only one primer of each set of four, i.e. the one that matches the target polynucleotide at the varying nucleotide, results in amplification.

The tag of each amplicon can then be labeled according to which of the four nucleotides was present in the primer (see e.g. column 13, lines 35-45). The labeled tags are then cleaved and hybridized to an array of tag complements, and the label (and thus the detected nucleotide) is detected for each position on the array.

The S primers in Brenner also contain a IIS restriction enzyme recognition site, so that, after a round of sequencing as described above, the end of the polynucleotide which has been sequenced can be cleaved off. See, for example, the set of primers at column 20, lines 5-15 of the patent. Because the position of the recognition site is constant with respect to the primer binding site, the enzyme will cleave at the same position in the target polynucleotide for each primer.

The Examiner's characterization of the Brenner reference

In the response filed August 9, 2006 (pages 11-12), applicants stated that the Examiner had mischaracterized the teachings of the Brenner reference with regard to, for example, step (b) (generating a size ladder) and step (e) (copying the labeled oligonucleotide tags) of applicants' claim 1. Because the rejection has been reproduced verbatim in the current Office Action, applicants again assert that these statements regarding the reference are inaccurate.

Contrary to the Examiners' statement at (b1) on page 5 of the present Office Action, Brenner does not teach "generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate" (step b of applicants' claim 1). As explained above, the enzymatic cleavage from the IIS recognition site in each S primer (using BbvI in the working Example) will cleave at the same position in the target polynucleotide for each primer, and thus will not create a "size ladder".

For the same reason, this shortening does not encompass "shortening by a different amount

said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount" (Examiner's statement at (b2), regarding dependent claim 2).

Even if this shortening did form a "size ladder" of some sort, the process would bear no relation in context to the applicants' claim as a whole. In Brenner, this shortening is carried out after a round of sequencing, so that the end of the polynucleotide which has been sequenced can be cleaved off: "In the shortening cycles, a predetermined number of previously identified nucleotides are cleaved from the target polynucleotides and the shortened polynucleotides are employed in the next cycle of nucleotide identification" (column 5, lines 6-12; see also column 14, lines 31-43). In the applicants' method, on the contrary, the size ladder is formed from each tag-polynucleotide conjugate before any labeling (step d) or sequence determination (steps d-f) takes place.

Nor does Brenner show "copying the labeled oligonucleotide tags of each polynucleotide fragment" (step e of applicants' claim 1), as asserted on page 6 of the Office Action, since in Brenner, the tags are labeled after they have been copied (*i.e.* selectively amplified) (see e.g. column 13, lines 35-45 and column 21, lines 23-28).

C. Analysis

Regarding motivation to combine, the Examiner suggests that one would be motivated to modify Brenner by "using the size selection of Wong", to "achieve a sampling frequency...useful for shotgun sequencing" (pages 11-12 of Office Action).

The passage pointed to in Wong (column 12, lines 6-16) stipulates the length of polynucleotide fragments (i.e., 400-2000 nucleotides) that should be used in a sequencing operation "to achieve a desired sampling frequency for effective shotgun sequencing". One skilled in the art would understand this passage to mean that sequencing the end regions of fragments of this length should provide a satisfactory representation of the original polynucleotide in "shotgun sequencing".

The passage pointed to in Brenner (column 11, lines 50-55) concerns the number of tag-polynucleotide conjugates that should be selected ("sampled") from a large population of tag-polynucleotide conjugates, in order to reduce the probability of different polynucleotides having the same tag ("doubles"), while still using a large enough sample of the original population of tag-

polynucleotide conjugates for "adequate coverage of a target polynucleotide in a shotgun sequencing operation."

The "sampling frequencies" referred to in these two passages, then, refer to entirely different concepts. Thus, the "size selection of Wong" (i.e. polynucleotide fragments 400-2000 nucleotides in length) would not be applicable to achieving "a sampling frequency" as taught in Brenner. The process of "sampling" in Brenner refers to selecting a sample of tag-polynucleotide conjugates from a larger population of tag-polynucleotide conjugates, and is not concerned with the length of the polynucleotides.

Moreover, even if the references were combined, the combination would not teach or suggest all the claim limitations. "In order to establish a *prima facie* case of obviousness, ... the prior art reference, or references when combined, must teach or suggest all the claim limitations" (MPEP §2143).

As discussed above, a distinguishing feature of applicants' claimed method is that tagged polynucleotides to be sequenced are processed into size ladders, and the resulting tagged fragments are then separated into size classes, before the step of "labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment" takes place. Such a feature is shown in neither of the cited references.

In view of the foregoing, the applicant respectfully requests the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

IV. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

Respectfully submitted,

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